

## Effect of Spaceflight on Ability of Monocytes To Respond to Endotoxins of Gram-Negative Bacteria<sup>▽</sup>

Indreshpal Kaur,<sup>1\*</sup> Elizabeth R. Simons,<sup>2</sup> Asha S. Kapadia,<sup>3</sup> C. Mark Ott,<sup>4</sup> and Duane L. Pierson<sup>4</sup>

*Enterprise Advisory Services Inc., Houston, Texas<sup>1</sup>; Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts<sup>2</sup>; School of Public Health, University of Texas Health Science Center, Houston, Texas<sup>3</sup>; and NASA Johnson Space Center, Houston, Texas<sup>4</sup>*

Received 5 February 2008/Returned for modification 28 March 2008/Accepted 21 August 2008

**Astronauts live and work in relatively crowded, confined environments on the Space Shuttle and the International Space Station. They experience a unique set of stressors that contribute to a diminishment of many immune responses. This study investigated the ability of the shuttle crew members' monocytes to respond to gram-negative endotoxin that they could encounter during infections. Blood specimens were collected from 20 crew members and 15 control subjects 10 days before launch, 3 to 4 h after landing, and 15 days after landing and from crew members during their annual medical examination at 6 to 12 months after landing. When challenged with gram-negative endotoxin, the crew member's monocytes collected at all three time points produced lower levels of interleukin-6 (IL-6) and IL-1 $\beta$  and higher levels of IL-1ra and IL-8 compared to those of control subjects. Cytokines were assessed by measuring the number of cells positive for intracellular cytokines. These values returned to normal 6 to 12 months after landing, except for IL-1ra, which was still higher (five- to sixfold) than in controls. This phenomenon was accompanied by an increased expression of Toll-like receptor 4 and decreased expression of CD14 on the crew members' monocytes at all time points. There were also increased levels of the lipopolysaccharide binding protein in the plasma of the crew members 3 to 4 h and 15 days after landing. This study shows that spaceflight-associated factors (in-flight and preflight) modulate the response of monocytes to gram-negative endotoxins.**

During spaceflight, astronauts are affected by many unusual factors, such as microgravity, radiation, physical and mental stresses, and isolation. Not only is spaceflight associated with in-flight factors influencing the astronauts, but even before launch, in preparation for the mission, the astronauts experience changes in circadian rhythms, rigorous training, and stresses of anticipation factors. Additionally, confinement of the crew during flight can and has resulted in the transfer of microorganisms between crew members (13, 26, 27). All of these factors are known to affect many physiological parameters of the astronauts, including the immune system (12, 17, 20, 31, 32, 41). Their effects include decreased functions of cells of the innate immune system, including neutrophils (14, 36), monocytes (15), and NK cells (17, 21). The innate immune system initiates the host defense against invading microbes by specific recognition mechanisms. The family of Toll-like receptors (TLRs) on monocytes have been shown to be involved in innate immune recognition in response to microbial antigens (1, 6, 38), such as lipopolysaccharide (LPS), or endotoxin, a major virulence factor of gram-negative bacteria (29). Tapping et al. (39) have shown that TLR4 is the predominant signaling receptor for LPS in human blood. In blood, LPS binds to LPS binding protein (LBP), which facilitates the transfer of LPS to CD14 molecules in the CD14-TLR4-MD2 complex on the cell membrane of monocytes (11). CD14 plays a key role in trans-

mitting LPS signals intracellularly, resulting in the production of cytokines by the monocytes (45). CD14 is also found in the serum as soluble CD14 (sCD14) and can interact directly with LPS through LBP and with CD14<sup>-</sup> cells like endothelial or epithelial cells to induce cytokine production (40). In the experiments reported here, we studied the effects of factors associated with spaceflight on the ability of monocytes to respond to LPS with respect to cytokine expression and expression of CD14, TLR4, LBP, and sCD14.

### MATERIALS AND METHODS

**Subjects.** This study included 17 male and 3 female astronauts participating in four shuttle missions lasting 10 to 13 days. The ages of the astronauts ranged from 38 to 51 years. The control subject group consisted of 11 males and 4 females, in the same age range as the astronauts, who were healthy individuals not associated with the stresses of spaceflight activities. The controls were part of the Johnson Space Center's test subject pool and go through an Air Force class III medical examination prior to participation. The study was approved by the Johnson Space Center's Committee for the Protection of Human Subjects.

**Sample collection.** As a part of each health assessment, blood samples were collected from all of the subjects in heparinized Vacutainer tubes (Becton Dickinson, Mountain View, CA) at 10 days before launch (L-10), 3 to 4 h after landing (R+0), 3 days after landing (R+3), and 15 days after landing (R+15) and during their annual medical examination (AME) at 6 to 12 months after the flight. Blood samples from control subjects were collected to mimic the L-10, R+0, R+3, and R+15 samples collected from the crew members.

To minimize variability, all of the reagents used in this study had the same lot number and were read on the same flow cytometer 24 h after processing by the same operator. The flow cytometer was calibrated with standard beads every time before the samples were read. These steps were implemented to ensure confidence in the results obtained, by necessity, on different days. We have shown that this approach produced excellent reproducibility ( $P < 0.001$ ) in terms of the fluorescence intensity that is the endpoint of all of the functional assays performed in this study. The interassay and intra-assay variabilities had coefficients of variation of less than 15%.

\* Corresponding author. Present address: Department of Stem Cell Transplantation and Cellular Therapy, Box 65, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Phone: (713) 563-4811. Fax: (713) 563-4825. E-mail: ikaur@mdanderson.org.

<sup>▽</sup> Published ahead of print on 3 September 2008.

**Staining for surface expression of CD14 and TLRs.** Whole blood (0.1 ml) was incubated with anti-CD14 (BD Biosciences, San Jose, CA) anti-TLR2, and anti-TLR4 antibodies (eBioscience, San Diego, CA) for 10 min at room temperature. Isotype-matched antibodies were used as controls for nonspecific binding. Red blood cells were lysed with FACS Lysing Solution (BD Biosciences, San Jose, CA), and the remaining leukocytes were fixed in 1% paraformaldehyde (Sigma, St. Louis, MO). The samples were read on a FACScalibur using CellQuest software, and 100,000 events were collected. Gates were set on the CD14<sup>+</sup> cells, and the expression of TLR2 and TLR4 was monitored.

**LPS stimulation.** One milliliter of whole blood was incubated for 4 h at 37°C and 7% CO<sub>2</sub> with either phosphate-buffered saline or 1 µg/ml *Escherichia coli* LPS (Sigma Chemical, St. Louis, MO) in the presence of 10 µg/ml brefeldin A (Sigma Chemical, St. Louis, MO). At the end of the incubation, 100 µl of the blood was dispensed into four tubes and incubated at room temperature for 10 min with anti-CD14–allophycocyanin. Red blood cells were lysed by incubating the samples with 2 ml FACS Lysing Solution (BD Biosciences) for 10 min and permeabilized by incubation for 10 min with 500 µl of FACS permeabilizing solution (BD Biosciences). Each tube was then incubated for 30 min at room temperature with one of the following sets of antibodies: immunoglobulin G1 (IgG1)–fluorescein isothiocyanate (FITC) and IgG1–phycoerythrin (PE), interleukin-6 (IL-6)–FITC and IL-8–PE, tumor necrosis factor alpha (TNF-α)–PE, or IL-1β–FITC and IL-1ra–PE (BD Biosciences). The cells were fixed with 1% paraformaldehyde and read on a FACScalibur using CellQuest software, and 100,000 events were collected. Gates were set on the CD14<sup>+</sup> cells, and the expression of the intracellular cytokines was measured in this population.

**sCD14.** sCD14 was measured in the plasma of samples obtained from the control subjects and crew members by using the sCD14 Quantikine enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems, Minneapolis, MN. It is a sandwich enzyme immunoassay that uses an anti-sCD14 monoclonal antibody as the capture antibody and an enzyme-linked polyclonal anti-sCD14 antibody as the detecting antibody.

**LBP.** LBP was measured in the plasma of controls and crew members with the ELISA kit from Cell Sciences, Canton, MA. The human LBP kit is a solid-phase sandwich ELISA. A monoclonal antibody specific for human LBP is used for capture, and a polyclonal antibody specific for human LBP is used as the detecting antibody.

**Statistical analysis.** For this study, we wanted to compare (i) the crew members to the control subjects, (ii) different sampling times within a group (crew members or controls), and (iii) sampling times between different groups, i.e., between the crew members and controls. For each measurement described above, a two-way analysis of variance with repeated measures was performed for values obtained at L–10, R+0, R+3, and R+15 by using SigmaStat software. Factor A was the group (controls and crew members), and factor B was the sampling time, which was the repeated measure for each subject. The Holm-Sidak test was used for multiple comparisons, including pairwise comparisons and comparisons within a group. Results were reported as statistically significant in cases where the *P* value was found to be less than 0.05 with a 95% confidence interval. The test was performed at a significance level of 0.05. The correlation ratio or  $\eta^2$  (eta squared) was calculated according to the method described by Pearson (25) and Cohen (7). The value of  $\eta^2$  indicates the amount of variability in the dependent variable that can be explained or accounted for by the independent variable. The values obtained at the AME for the crew members were compared to the aggregated values for the controls at L–10, R+0, and R+3 by a one-way analysis of variance. We used the pattern of results over the different functions to point to a meaningful conclusion about the overall effect of spaceflight on cell functions in astronauts.

## RESULTS

**TLRs.** We determined the expression of TLR4 and TLR2 on the surface of monocytes from crew members and control subjects at L–10, R+0, and R+15 and during the AME of the crew members (Fig. 1). As we were not able to get enough of a blood sample from the crew members at R+3, we were unable to measure TLR expression at R+3. We observed a main effect of the group on TLR4 expression, such that compared to controls, all crew members showed an increase in TLR4 expression at all time points ( $P = 0.04, 0.03$ , and  $0.004$ , respectively,  $F = 12.79$  [degrees of freedom (df) = 1 and 17], and  $\eta^2 = 0.14$ ). The effect of different levels of group does not

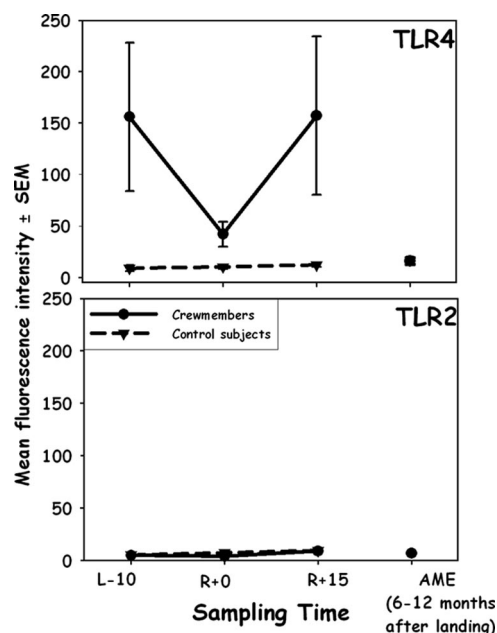


FIG. 1. Expression of the TLR4 and -2 on the monocytes of astronauts and crew members at L–10, R+0, R+15, and the AME. SEM, standard error of the mean.

depend on the level of sampling times. The expression of TLR4 on the astronauts' monocytes was significantly lower at R+0 compared to the values at L–10 and R+15. At the AME, the levels of TLR4 on crew members' monocytes were the same as the control subject levels, which did not vary significantly over the sampling times. Due to the large range of TLR4 at L–10 and R+15, the expression of TLR4 on crew members' monocytes also did not differ significantly between these two sampling times.

We did not see a main effect of the group on the expression of TLR2, as it did not differ significantly between controls and crew members ( $P = 0.127$ ,  $F = 2.5$  [df = 1 and 17], and  $\eta^2 = 0.032$ ). In addition, no significant difference in the expression of TLR2 was observed at different sampling times for either the control group or crew members. The effect of different levels of the group depends on the sampling time.

**Expression of CD14.** We observed a main effect of the group on the expression of CD14 such that the monocytes of crew members expressed significantly lower levels of CD14 compared to those of controls at L–10, R+0, R+3, and R+15 ( $P < 0.001$ ,  $F = 76.48$  [df = 1 and 33], and  $\eta^2 = 0.435$ ) (Fig. 2). We were not able to get enough blood samples from the crew members at R+15 and were not able to measure the expression of CD14 on the crew members' monocytes at R+15. The effect of different levels of group does not depend on the level of sampling times. However, 6 to 12 months after landing, during their AME, the expression of CD14 on the crew members' monocytes returned to levels similar to those of controls. There was no difference in the expression of CD14 on the monocytes of control subjects over the different sampling times, as was also observed in the case of the crew members.

**Intracellular cytokines (Fig. 3).** (i) **IL-6.** We did not see a main effect of the group on IL-6. The IL-6 expression in mono-

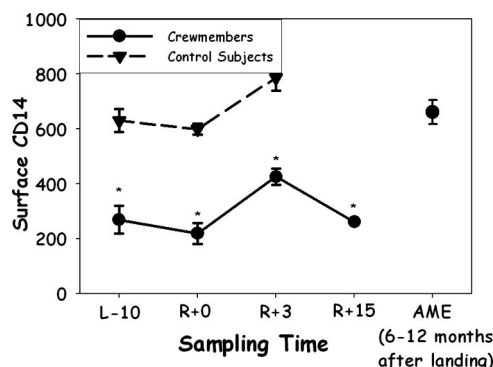


FIG. 2. Expression of CD14 (mean fluorescence intensity) on the monocytes of astronauts and crew members at L-10, R+0, R+3, R+15, and the AME. An asterisk indicates that the values for the crew members are significantly different from the control values.

cytes of crew members and controls differed over time, with crew members' values showing lower expression at L-10 and R+0 and not other time points ( $P < 0.01$  and  $0.04$ , respectively,  $F = 17.37$  [df = 1 and 33], and  $\eta^2 = 0.15$ ). At other sampling times (R+3, R+15, and the AME), the level of IL-6 produced by monocytes of crew members was similar to that of controls. When comparing the IL-6 produced by monocytes of crew members at different sampling times, there was a significant difference between the levels of IL-6 at R+0 and L-10 and at R+3 and L-10 ( $P = 0.006$  and  $0.003$ ,  $F = 4.06$  [df = 2 and 66], and  $\eta^2 = 0.058$ ). There was no difference between levels of IL-6 at R+0 and R+3. The amount of IL-6 produced by the monocytes of control subjects in response to LPS did not vary significantly between different sampling times.

(ii) **IL-8.** The effect of different levels of group was dependent on the sampling time. IL-8 expression on monocytes of crew members and controls differed over time, with crew members showing greater expression at R+0 and R+3 ( $P = 0.004$ ,  $F = 2.64$  [df = 1 and 33], and  $\eta^2 = 0.036$ ) and not other time points. At L-10, R+15, and the AME, the astronauts' monocytes produced levels of IL-8 in response to LPS comparable to those of control subjects. Looking at the time effect for crew members, the levels of IL-8 produced by the monocytes of crew members changed significantly between L-10 and R+0 ( $P = 0.002$ ), L-10 and R+3 ( $P = 0.021$ ), R+0 and R+15 ( $P = 0.01$ ), and R+3 and R+15 ( $P = 0.014$ ). That is, the IL-8 values at landing and 3 days after landing were significantly higher than those at L-10 and R+15. The control subjects produced similar levels of IL-8 in response to LPS at various sampling times.

(iii) **IL-1ra.** We observed a main effect of the group on IL-1ra, such that the monocytes of crew members produced significantly higher IL-1ra levels than those of controls at all time points ( $P < 0.001$ ,  $F = 74.5$  [df = 1 and 33], and  $\eta^2 = 0.39$ ). The level of IL-1ra produced by the crew members' monocytes was lowest at L-10 and did not return to the baseline, even 6 to 12 months after landing (at the AME). The monocytes of control subjects produced similar amounts of the cytokine IL-1ra at different sampling times in response to LPS.

(iv) **IL-1 $\beta$ .** IL-1 $\beta$  expression on monocytes of crew members and controls differed over time, with crew members producing lower IL-1 $\beta$  levels at L-10, R+0, and R+3 and not at other

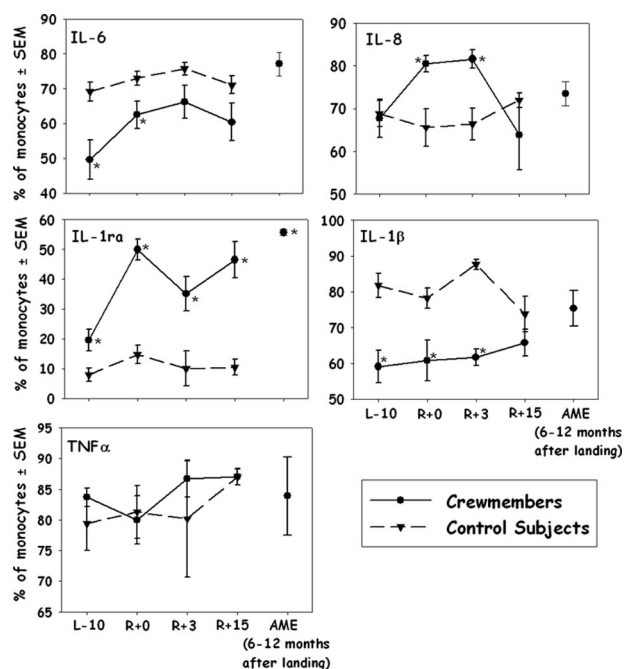


FIG. 3. LPS-stimulated alterations in the number of cells expressing intracellular cytokines among monocytes collected from crew members and control subjects at L-10, R+0, R+3, and R+15 and from crew members at the AME. The response is expressed in terms of the number of monocytes expressing the particular cytokine. An asterisk indicates that the values for the crew members are significantly different from the control values. SEM, standard error of the mean.

time points ( $P = <0.001$ ,  $0.005$  and  $0.007$ , respectively,  $F = 21.23$  [df = 1 and 33], and  $\eta^2 = 0.23$ ). At R+15, the crew members' monocytes were able to produce normal levels of IL-1 $\beta$  and did so at the AME also. There was no significant difference in the amounts of IL-1 $\beta$  produced by the crew members' monocytes at L-10, R+0, and R+3.

(v) **TNF- $\alpha$ .** For TNF- $\alpha$ , we did not observe any main effect, such that there were no differences in the levels of TNF- $\alpha$  produced by the controls and crew members at any of the time points tested in response to LPS ( $P = 0.858$ ,  $F = 0.03$  [df = 1 and 33], and  $\eta^2 = 0.0003$ ).

**sCD14.** sCD14 levels were measured in the plasma of controls and crew members. We did not observe a main effect for the group, as there was no difference in the levels of sCD14 present in the plasma of controls and crew members at any of the sampling times studied (data not shown).

**LBP.** Plasma LBP levels of crew members and controls differed over time, with crew members showing greater expression at R+0 and R+15 and not other time points, such that at R+0 and R+15, there was an increase in the LBP levels in the plasma of crew members ( $P = 0.002$ ,  $F = 4.79$  [df = 1 and 33], and  $\eta^2 = 0.08$ ) and the levels returned to the baseline at the AME (Fig. 4). Even at L-10, the LBP levels in the plasma of crew members were similar to those of controls. The mean LBP level in the plasma of controls was  $14.5 \mu\text{g/ml}$ , and there was no statistically significant difference in the values at the three sampling times. Plasma samples from crew members at R+3 were not available.

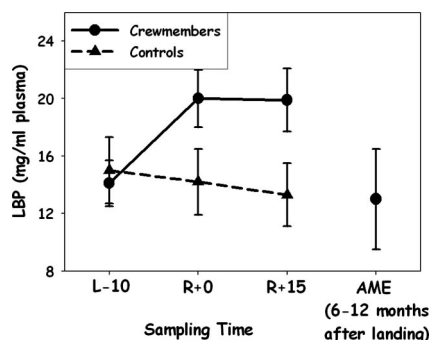


FIG. 4. LBP levels in plasma collected from crew members and control subjects at L-10, R+0, and R+15 and from crew members at the AME.

**Effects of gender, age, and other factors.** Although the sample numbers were small, special attention was given to statistical analyses of all of the data to look for trends based on gender and other factors. Statistical analysis of the data showed no association of the changes observed with age, gender, mission duration, flight experience, mission role (such as pilot), or flight event (such as a space walk).

## DISCUSSION

Our previous studies have shown that spaceflight alters the functions of both neutrophils and monocytes, leading to a decreased ability to phagocytize bacteria and generate an oxidative burst (14, 15). The functions of the crew members' monocytes and neutrophils were altered even at L-10, validating our finding that L-10 was not a suitable baseline time point. Similar findings have been reported by Stowe et al. (35), who observed a significant increase in Epstein-Barr virus capsid antigen antibodies and a decrease in anti-Epstein-Barr virus nuclear antigen antibodies in astronauts at L-10. In the weeks prior to a flight, the crew members go through rigorous training, medical examinations, and isolation during the last 10 days, when their circadian rhythm may be adjusted to the flight schedule. Anticipation before the flight plus other physiological and psychological factors can cause modulations in the immune system. Thus, for this study, we collected blood samples from crew members 6 to 12 months after a spaceflight, during their AMEs. Monocytes obtained during this sampling time displayed functional levels very similar to those of low-stress control subjects in all cases, except for the increased expression of IL-1ra in response to LPS, which did not return to baseline levels, even at the AME. These functional levels were used as baseline (low-stress) values. Also, the whole-blood assays used in this study rule out the chance of activating the monocytes, as is seen when purified monocytes are used for experiments (34).

There have been various reports of increases or decreases in the number of monocytes after a spaceflight (20, 22, 37, 42). Similar to our findings of decreased expression of CD14 on the surface of the crew members' monocytes, Crucian et al. reported no change in the absolute number of monocytes but saw a decrease in the percentage of CD14<sup>+</sup> CD16<sup>+</sup> cells (8). The decreased expression of CD14 and the increased expression of

TLR4 on the monocytes of crew members in our present study may be the cause of altered responsiveness of the crew members' monocytes to LPS, as LPS responsiveness is dependent on the physical association of the LPS/CD14-TLR4-MD2 complex, without which signal transduction does not occur (10, 23). Bihl et al. (3) have shown that transgenic mice overexpressing TLR4 have a survival advantage early during infection, as these mice lived longer after salmonella infection. However, these mice also developed an excessive inflammatory response to LPS during the later stages of infection. Dziarski and Gupta (9) have shown that when monocytes were stimulated with gram-negative or gram-positive cell wall components, the IL-8 gene was the most strongly induced chemokine gene. This involves the association of MD2 with both TLR2 and TLR4. The increased expression of IL-8 seen in the astronauts at R+0 and R+3 could be a result of signaling through the TLR4 transduction cascade. IL-8, a chemoattractant cytokine, can then induce migration of leukocytes from the circulation to tissues (30). Altered responses of crew members' monocytes to LPS could also be a result of increased levels of LBP in their plasma. Acute-phase LBP has been shown to have a protective effect against LPS and bacterial infections and may represent a defense mechanism against infections (18, 44). LBP provides a shift in the balance of the LPS-directed dual actions of the protein disfavoring proinflammatory cell activation and clearance of LPS via major cellular and systemic pathways by binding to high-density lipoprotein complexes (44). Thus, the increased expression of TLR4 and LBP in crew members may be a pathway their immune systems adopt while adapting to the spaceflight environment. While increased levels of sCD14 have been shown to decrease the monocytes' response to LPS by transferring cell-bound LPS to plasma lipoproteins (16), the lack of change in sCD14 in these crew members suggests that this is not a likely mechanism.

Spaceflight also altered the ability of the crew members' monocytes to produce cytokines in response to LPS. IL-1 is an important cytokine of the innate immune system, and it regulates the functions of the adaptive immune system (2). Increased levels of IL-1 lead to inflammation, stimulation of cells, and initiation of the transcription of proinflammatory genes for IL-6 and IL-8. The balance between IL-1 $\beta$  and its receptor antagonist IL-1ra influences the possible development of inflammation. Under normal physiological conditions, high levels of IL-1ra are produced to functionally inhibit the biological effects of IL-1. This also occurred in the crew members in this study. The increased expression of IL-8, a chemoattractant for neutrophils, results in increased demargination of the neutrophils. Chapes et al. (5) observed monocytopenia in rats aboard a shuttle mission lasting 8 days. Resident macrophages of these rats secreted higher TNF- $\alpha$  levels in response to LPS and staphylococcal enterotoxin B compared to the ground controls. Also, monocytes of rats subjected to simulated weightlessness showed a decrease in the expression of TNF- $\alpha$  in response to LPS (43). After exercise, monocytes produced less TNF- $\alpha$  and IL-1 $\alpha$  in response to LPS (33). Monocytes of subjects exposed to a cold environment also produced less TNF- $\alpha$  and IL-1 $\beta$  and increased amounts of IL-6 and IL-1ra (28). Similar effects were seen in elderly humans, whose monocytes produced decreased levels of IL-1 $\beta$  and TNF- $\alpha$  in response to LPS (4). The effects observed in the



astronauts are different than those observed in the above-mentioned models, suggesting that factors associated with spaceflight are different than those influencing other models. Spaceflight is a unique stress model impacted consistently or intermittently by myriad stresses, including anxiety, psychosocial conflict, physical stress (e.g., spacewalks), high g forces at launching and landing, sleep deprivation, increased radiation, and physiological changes (decreased plasma volume, shifts in body fluids, and altered pharmacodynamics). Complete duplication of stressors that can be encountered during spaceflight cannot be duplicated in ground models. It is also difficult to say which factors were responsible for which changes seen in the functions of the crew members' monocytes, as many factors exert their effects simultaneously. Also, as the stress hormones are dependent on circadian rhythms and the landing time is different for each shuttle mission, the stress hormone levels in the samples obtained after landing are not a true representation of the stress level, and hence we did not report the levels of stress hormones. Furthermore, in this and our previous studies (14, 15), we found that prior experience, the type of in-flight activity, gender, or age did not influence the intensity of changes observed in the immune functions. In one of our previous studies, we showed that the modulation in the functions of neutrophils was dependent on the length of the mission (14), whereas the functions of the monocytes were not (15).

Nickerson et al. (24) and Lynch et al. (19) have shown that microorganisms cultured in microgravity analogs increase their resistance to several environmental stressors, such as high osmolarity and high acidity. Nickerson et al. (24) observed that the enhanced virulence of *Salmonella enterica* serovar Typhimurium grown under modeled microgravity did not involve increased expression of many genes implicated in the virulence of this bacterium. Rather, many genes known to be involved in virulence were expressed at a lower level, including genes involved in LPS production. *S. enterica* serovar Typhimurium grown under modeled microgravity also possessed about half as much LPS as the controls. These studies, along with the findings of our present study, indicate that further research into both host and pathogen responses needs to be conducted to determine if the risk of infectious disease may be altered during spaceflight.

Taken together, the present study and our previous descriptions of human neutrophil and monocyte function modulation by spaceflight (14, 15) indicate that there is indeed a modulation of crew members' phagocytes' responses to a bacterial or endotoxin challenge. Since these may affect the crew members' ability to fight infections and/or heal from any wounds sustained during their time in space, the mechanisms of this impairment and possible ways to counteract the latter need further exploration.

#### ACKNOWLEDGMENTS

We extend our appreciation to the astronauts and control subjects for participating in this study.

We thank the National Aeronautics and Space Administration for providing funding through NRA 111-30-10-06 to conduct this work.

#### REFERENCES

- Anderson, K. V. 2000. Toll signaling pathways in the innate immune response. *Curr. Opin. Immunol.* **12**:13–19.
- Arend, W. P. 2002. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* **13**:323–340.
- Bihl, F., L. Salez, M. Beaubier, D. Torres, L. Lariviere, L. Laroche, A. Benedetto, D. Martel, J. M. Lapointe, B. Ryffel, and D. Malo. 2003. Overexpression of Toll-like receptor 4 amplifies the host response to lipopolysaccharide and provides a survival advantage in transgenic mice. *J. Immunol.* **170**:6141–6150.
- Bruunsgaard, H., A. N. Pedersen, M. Schroll, P. Skinhoj, and B. K. Pedersen. 1999. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clin. Exp. Immunol.* **118**:235–241.
- Chapes, S. K., S. J. Simske, G. Sonnenfeld, E. S. Miller, and R. J. Zimmerman. 1999. Effects of spaceflight and PEG-IL-2 on rat physiological and immunological responses. *J. Appl. Physiol.* **86**:2065–2076.
- Chow, J. C., D. W. Young, D. T. Golenbock, W. J. Christ, and F. Gusovsky. 1999. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **274**:10689–10692.
- Cohen, J. 1973. Eta-squared and partial eta-squared in fixed factor ANOVA designs. *Educ. Psychol. Measurements* **33**:107–112.
- Crucian, B. E., M. L. Cabbage, and C. F. Sams. 2000. Altered cytokine production by specific human peripheral blood cell subsets immediately following space flight. *J. Interferon Cytokine Res.* **20**:547–556.
- Dziarski, R., and D. Gupta. 2000. Role of MD-2 in TLR2- and TLR4-mediated recognition of gram-negative and gram-positive bacteria and activation of chemokine genes. *J. Endotoxin Res.* **6**:401–405.
- Fitzgerald, K. A., D. C. Rowe, and D. T. Golenbock. 2004. Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes Infect.* **6**:1361–1367.
- Heumann, D., and T. Roger. 2002. Initial responses to endotoxins and gram-negative bacteria. *Clin. Chim. Acta* **323**:59–72.
- Huntoon, C. L., P. C. Johnson, and N. M. Cintron. 1993. Hematology, immunology, endocrinology and biochemistry, p. 222–239. *In* A. E. Nicogossian, C. L. Huntoon, and S. L. Pool (ed.), *Space physiology and medicine*, 2nd edition. National Aeronautics & Space Administration, Washington, DC.
- Kass, E. H. 1971. Resistance to infections in extended space flight. *Life Sci. Space Res.* **9**:35–41.
- Kaur, I., E. R. Simons, V. A. Castro, C. M. Ott, and D. L. Pierson. 2004. Changes in neutrophil functions in astronauts. *Brain Behav. Immun.* **18**:443–450.
- Kaur, I., E. R. Simons, V. A. Castro, C. M. Ott, and D. L. Pierson. 2005. Changes in monocyte functions of astronauts. *Brain Behav. Immun.* **19**:547–554.
- Kitchens, R. L., P. A. Thompson, S. Viriyakosol, G. E. O'Keefe, and R. S. Munford. 2001. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. *J. Clin. Investig.* **108**:485–493.
- Konstantinova, I. V., M. P. Rykova, A. T. Lesnyak, and E. A. Antropova. 1993. Immune changes during long-duration missions. *J. Leukoc. Biol.* **54**:189–201.
- Lamping, N., R. Dettmer, N. W. Schroder, D. Pfeil, W. Hallatschek, R. Burger, and R. R. Schumann. 1998. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *J. Clin. Investig.* **101**:2065–2071.
- Lynch, S. V., E. L. Brodie, and A. Matin. 2004. Role and regulation of sigma S in general resistance conferred by low-shear simulated microgravity in *Escherichia coli*. *J. Bacteriol.* **186**:8207–8212.
- Meehan, R. T., L. S. Neale, E. T. Kraus, C. A. Stuart, M. L. Smith, N. M. Cintron, and C. F. Sams. 1992. Alteration in human mononuclear leucocytes following space flight. *Immunology* **76**:491–497.
- Mehta, S. K., I. Kaur, E. A. Grimm, C. Smid, D. L. Feedback, and D. L. Pierson. 2001. Decreased non-MHC-restricted (CD56<sup>+</sup>) killer cell cytotoxicity after spaceflight. *J. Appl. Physiol.* **91**:1814–1818.
- Mills, P. J., J. V. Meck, W. W. Waters, D. D'Aunno, and M. G. Ziegler. 2001. Peripheral leukocyte subpopulations and catecholamine levels in astronauts as a function of mission duration. *Psychosom. Med.* **63**:886–890.
- Miyake, K. 2004. Innate recognition of lipopolysaccharide by Toll-like receptor 4-MD-2. *Trends Microbiol.* **12**:186–192.
- Nickerson, C. A., C. M. Ott, J. W. Wilson, R. Ramamurthy, C. L. LeBlanc, K. Honer zu Bentrop, T. Hammond, and D. L. Pierson. 2003. Low-shear modeled microgravity: a global environmental regulatory signal affecting bacterial gene expression, physiology, and pathogenesis. *J. Microbiol. Methods* **54**:1–11.
- Pearson, K. 1911. On a correction needful in the case of the correlation ratio. *Biometrika* **8**:254–256.
- Pierson, D. L., M. Chidambaram, J. D. Heath, L. Mallary, S. K. Mishra, B. Sharma, and G. M. Weinstock. 1996. Epidemiology of *Staphylococcus aureus* during space flight. *FEMS Immunol. Med. Microbiol.* **16**:273–281.
- Pierson, D. L., S. K. Mehta, B. Magee, and S. K. Mishra. 1995. Person-to-person transfer of *Candida albicans* in the spacecraft environment. *J. Med. Vet. Mycol.* **33**:145–150.
- Rhind, S. G., J. W. Castellani, I. K. Brenner, R. J. Shephard, J. Zamecnik,

- S. J. Montain, A. J. Young, and P. N. Shek. 2001. Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**:R66–R75.
29. Rietschel, E. T., T. Kirikae, F. U. Schade, U. Mamat, G. Schmidt, H. Loppnow, A. J. Ulmer, U. Zahringer, U. Seydel, F. Di Padova, M. Schreier, and H. Brade. 1994. Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J.* **8**:217–225.
30. Smith, C. W. 1993. Leukocyte-endothelial cell interactions. *Semin. Hematol.* **30**:45–55.
31. Sonnenfeld, G. 2001. Extreme environments and the immune system: effects of spaceflight on immune responses. *J. Allergy Clin. Immunol.* **107**:19–20.
32. Sonnenfeld, G., A. D. Mandel, I. V. Konstantinova, G. R. Taylor, W. D. Berry, S. R. Wellhausen, A. T. Lesnyak, and B. B. Fuchs. 1990. Effects of spaceflight on levels and activity of immune cells. *Aviat. Space Environ. Med.* **61**:648–653.
33. Starkie, R. L., D. J. Angus, J. Rolland, M. Hargreaves, and M. A. Febbraio. 2000. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *J. Physiol.* **528**: 647–655.
34. Stent, G., and S. M. Crowe. 1997. Effects of monocyte purification and culture on integrin expression. *APMIS* **105**:663–670.
35. Stowe, R. P., D. L. Pierson, and A. D. Barrett. 2001. Elevated stress hormone levels relate to Epstein-Barr virus reactivation in astronauts. *Psychosom. Med.* **63**:891–895.
36. Stowe, R. P., C. F. Sams, S. K. Mehta, I. Kaur, M. L. Jones, D. L. Feedback, and D. L. Pierson. 1999. Leukocyte subsets and neutrophil function after short-term spaceflight. *J. Leukoc. Biol.* **65**:179–186.
37. Stowe, R. P., C. F. Sams, and D. L. Pierson. 2003. Effects of mission duration on neuroimmune responses in astronauts. *Aviat. Space Environ. Med.* **74**: 1281–1284.
38. Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda, and S. Akira. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* **11**:443–451.
39. Tapping, R. I., S. Akashi, K. Miyake, P. J. Godowski, and P. S. Tobias. 2000. Toll-like receptor 4, but not Toll-like receptor 2, is a signaling receptor for Escherichia and Salmonella lipopolysaccharides. *J. Immunol.* **165**:5780–5787.
40. Tapping, R. I., and P. S. Tobias. 2000. Soluble CD14-mediated cellular responses to lipopolysaccharide. *Chem. Immunol.* **74**:108–121.
41. Taylor, G. R. 1993. Overview of spaceflight immunology studies. *J. Leukoc. Biol.* **54**:179–188.
42. Taylor, G. R., L. S. Neale, and J. R. Dardano. 1986. Immunological analyses of U.S. Space Shuttle crewmembers. *Aviat. Space Environ. Med.* **57**:213–217.
43. Wang, T., G. Yang, P. Hu, and X. Wen. 1997. Effect of simulated weightlessness on TNF-alpha production and the response of bone marrow cells to GM-CSF in rats. *Space Med. Med. Eng. (Beijing)* **10**:168–171.
44. Wurfel, M. M., S. T. Kunitake, H. Lichenstein, J. P. Kane, and S. D. Wright. 1994. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J. Exp. Med.* **180**:1025–1035.
45. Ziegler-Heitbrock, H. W., and R. J. Ulevitch. 1993. CD14: cell surface receptor and differentiation marker. *Immunol. Today* **14**:121–125.